The Oncologist
Physician Education

Expansion of Immunostimulatory Dendritic Cells from Peripheral Blood of Patients with Cancer

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INTRODUCTION

Clinical investigators are keenly interested in the role of antigen-presenting cells (APCs) in the initiation of immune responses because of potential use of APCs in immune cell therapy. Pioneer studies in mammals by *Steinman* and coworkers [1] have demonstrated that the specialized system of APCs is constituted by bone marrow-derived dendritic cells. Dendritic cells are distinguished by their unique ability to capture, process, and present antigens into peptide-HLA complexes to naive T lymphocytes and to deliver the costimulatory signal necessary for T-lymphocyte activation. In this article, we summarize the main experimental evidence supporting the hypothesis that individuals vaccinated with manipulated dendritic cells can mount tumor-specific humoral and cellular responses. This can lead to tumor regression as well as protective immunity to tumor growth in vivo [2].

IDENTIFICATION OF DENDRITIC CELLS

Dendritic cells are leukocytes derived from hematopoietic stem cells along the myeloid differentiation pathway; mature dendritic cells are specialized for antigen presentation to the immune system (Fig. 1). In humans, dendritic cells circulate in the peripheral blood and are found in virtually all tissues of the body where, depending on the location, they are referred to as interstitial dendritic cells (heart, kidney, gut, lung), Langerhans cells (skin, mucous membrane), interdigitating dendritic cells (thymic medulla, secondary lymphoid tissue), or veiled cells (lymph, blood) [3]. Dendritic cells can be identified by their morphology and cell-surface membrane phenotype, and by assays measuring proliferation of allogeneic as well as autologous resting naive T lymphocytes.

Dendritic cells (Fig. 2), whether examined by phase contrast, light, or transmission electron microscopy, are characterized by a villous cell surface with branching projections, a lobulated nucleus, large Golgi apparatus, and multivesicular bodies occasionally in continuity with the membrane complexes. An intracytoplasmic hallmark of cutaneous Langerhans cells are the Birbeck granules, rod-shaped organelles with a double membrane and a cross-striated pattern, sometimes terminating in a small vesicle giving an overall tennis-racket appearance. The function of these granules is not clear, although an association with endosomal

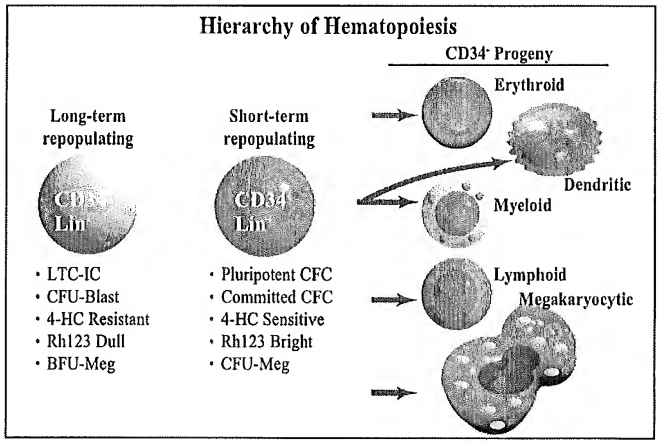

Figure 1. Hierarchy of hematopoiesis.

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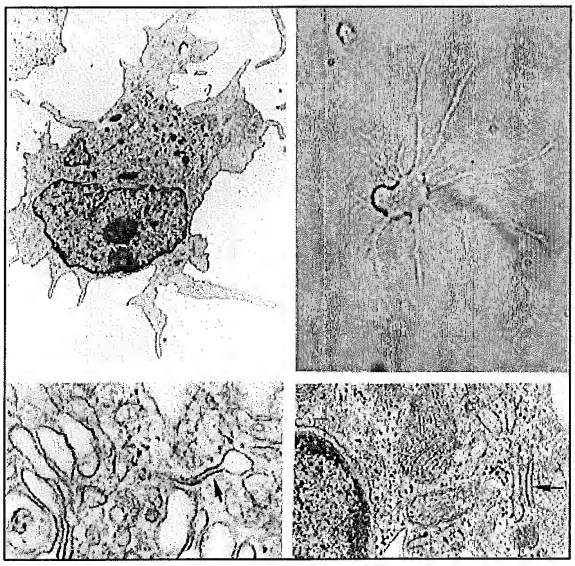

Figure 2. Photomicrographs of dendritic cells. Reprinted with permission from [5].

structures has been suggested [4]. Intriguingly, Birbeck granules are found in dendritic cells generated ex vivo from $CD34^+$ hematopoietic progenitor cells, but not in dendritic cells generated from monocytes [3, 5].

Cutaneous Langerhans cells, as well as most of the dendritic cells generated ex vivo from human $CD34^+$ progenitor cells, express CD1a antigen [3,5]. Although CD1a can be found on cortical thymocytes and some B lymphocytes, its presence (noted by immunofluorescence and flow cytometry) is the most useful way to quantitate the ex vivo generation of dendritic cells. Furthermore, dendritic cells express peculiarly high levels of class I and II histocompatibility complex structures, costimulatory molecules for T lymphocytes such as B7-1 (CD80) and B7-2 (CD86), and adhesion molecules such as ICAM-1 (CD54) and ICAM-3 (CD50) involved in dendritic cell-dependent T-lymphocyte proliferation. Dendritic cells lack the monocyte/macrophage lineage- and lymphocyte lineage-restricted antigens with the exception of the CD4 antigen [5]. Relevant costimulatory (B7-1 and B7-2) and adhesion molecules (ICAM-1) are expressed on all $CD1a^+$ cells derived from $CD34^+$ progenitor cells, but on fewer $CD1a^+$ cells derived from monocytes (Table 1) [6].

Table 1. Phenotype of $CD1a^+$ dendritic cells generated from mobilized $CD34^+$ progenitors and monocytes

	$CD1a^+$ cells derived from	
Antigens	**$CD34^+$ progenitors**	**Monocytes**
CD14	2%	3%
CD80 (B7-1)	100%	84%
CD86 (B7-2)	100%	67%
CD54 (ICAM-1)	100%	67%
Class I		
HLA-A 0201	100%	100%
Class II		
HLA-DR	100%	100%
HLA-DQ	60%	89%

Dendritic Cells for Experimental Antitumor Cell Therapy

The goal of vaccination is induction of protective immunity. Originally, vaccinations were used in the setting of infectious diseases, but now have been expanded to include the treatment of allergy, autoimmune diseases, and tumors. A rational approach to vaccination involves the identification of the protective effector mechanism, the choice of an antigen that can induce a response in all individuals, and the use of an appropriate delivery system to induce the correct response [2].

Some tumor cells are antigenic in the sense that they express tumor-associated antigens recognizable by T lymphocytes in a syngeneic host. The tumor cells are also poorly immunogenic because they lack the cellular armamentarium for specific T-lymphocyte recognition, activation, and costimulation possessed by both APCs and dendritic cells.

There are at least two approaches to tumor vaccination: identifying a tumor-associated antigen to be used as a vaccine, and increasing the immunogenicity of tumor cells to allow the immune system to decide which antigen to attack (Fig. 3). In experimental models with the appropriate manipulation, the immune system has the ability to mount responses that can destroy tumor cells [7-14].

Given the richness of recently identified tumor-associated antigens and their corresponding peptide epitopes recognized by MHC-restricted $CD8^+$ or $CD4^+$ T lymphoctyes (Table 2), investigators are currently evaluating the clinical efficacy of specific tumor-associated antigen-based vaccines for the treatment of various malignancies. In a cooperative clinical trial [15], we have observed partial tumor regressions in patients with $HLA\text{-}A1^+$ melanoma treated with a naked MAGE 3 peptide epitope vaccine. In this regard, one may hypothesize that the effectiveness of peptide-based vaccines will be improved with the administration of appropriate adjuvants capable of promoting cellular immunity. In another pioneer clinical trial, the ability of autologous monocyte-derived dendritic cells pulsed ex vivo with non-Hodgkin's lymphoma-specific idiotype protein to stimulate host immunity when infused as a vaccine was shown [16]. In this study, active immunotherapy of patients with B-cell lymphoma against idiotypic determinants led to antitumor immunity correlating with improved clinical outcome in some of the patients.

Regardless of the type of immune-cell manipulation, i.e., ex vivo pulsing with tumor-associated antigen peptides or

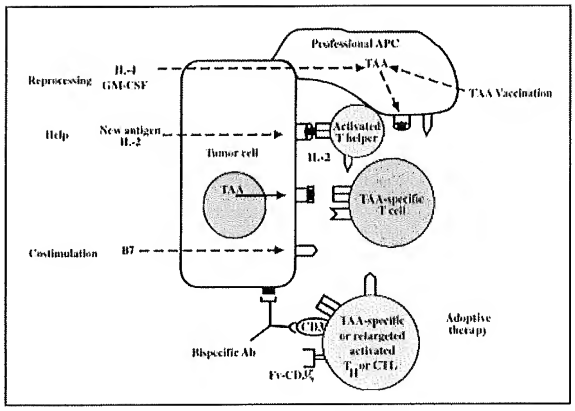

Figure 3. Possible ways to boost the T-cell response to a tumor-associated antigen. (Modified from [2]).

induction with tumor-associated antigen genes, the clinical success of the technique depends on the ability to generate large numbers of functional dendritic cells.

Ex Vivo Expansion of Dendritic Cells

Although dendritic cells circulate in the peripheral blood and are found in nearly all tissues of the body, it is difficult to obtain enough dendritic cells for ex vivo manipulation because of their scattered locations and low frequency in the blood, where they compose approximately 0.1% of all leukocytes. It has been learned, however, that tumor-necrosis factor-alpha (TNF-α) cooperates with granulocyte-macrophage colony-stimulating factor (GM-CSF) to generate dendritic cells from $CD34^+$ hematopoietic progenitor cells harvested from bone marrow, cord blood, or peripheral blood [4, 5, 17-24]. Interleukin (IL)-4 also cooperates with GM-CSF in the ex vivo generation of dendritic cells from circulating monocytes [20, 25]. The methods used to convert human myeloid cells into dendritic cells are summarized in Table 3. Three considerations should be used in evaluating these methods in regard to clinical use of ex vivo-generated dendritic cells: A) the type of dendritic cells generated from either monocytes or $CD34^+$ hematopoietic progenitor cells; B) the sources of the serum used; and C) the combination of the cytokines needed for optimal ex vivo expansion of functional immunostimulatory dendritic cells.

Table 2. Examples of tumor antigens capable of eliciting T cell responses

Activated oncogene products	
• Mutated	Position 12 point mutation of $p21^{ras}$
• Rearranged	bcr-abl
• Overexpressed	HER-2/neu
Tumor suppressor gene products	*p53* mutations
Reactivated embryonic gene products	MAGE family (at least 12 genes) BAGE GAGE
Melanocyte differentiations antigens	Tyrosinase protein Melan-A/MART1 gp100 gp75
Viral gene products	Human Papilloma Virus antigens (E8 & E7) Epstein Barr Virus EBNA-1 gene products
Idiotypic epitopes	Ig and TCR hypervariable regions

Dendritic cells derived from monocytes are being used in phase 1 and phase 2 clinical trials in patients with advanced-stage malignancies. The purpose of these trials is to evaluate the toxicity and immune response in patients administered subcutaneous injections of dendritic cells pulsed ex vivo with melanoma tumor-associated antigen peptides [26, 27] or B-cell lymphoma idiotype protein [16]. Early reports indicate that patients who are $HLA\text{-}A1^+$ and whose melanoma cells express MAGE-1 have not had any toxicity from the injections. The injections can induce peptide-specific, autologous melanoma-reactive $CD8^+$ cytotoxic lymphocyte responses at both the vaccination site and at distant tumor sites, as well as in the peripheral blood [27].

Our group has determined the optimal method for obtaining a large number of functional dendritic cells from $CD34^+$ progenitor cells harvested from patients with cancer [5]. Cells used in peripheral progenitor cell transplantation in trials of high-dose sequential chemotherapy for the treatment of breast cancer [28] or non-Hodgkin's lymphoma [29] have been shown to contain the $CD34^+$ cells that produce dendritic cells. These ex vivo-expanded dendritic cells have the characteristics of professional APCs: typical dendritic cell morphology; expression of surface membrane CD1a antigen and high levels of CD80, CD86, CD54, HLA class I and antigens, as well as lack of macrophage-restricted CD14 antigen; the capacity to induce the proliferation of allogeneic T cells in primary mixed leukocyte reaction; and the capacity to process and present antigens to T lymphocytes. Limiting dilution assays of $CD34^+$ cells selected from leukapheresis packs showed that progenitors of dendritic cells were approximately 140-fold more numerous compared with cells normally in the peripheral blood.

A systematic search was done to determine the best culture conditions capable of producing the maximum number of dendritic cells ex vivo, using a variety of exogenous stimuli as well as monocyte-derived and $CD34^+$-derived dendritic cells [30]. The generation of dendritic cells from $CD34^+$ mobilized cells by TNF-α and GM-CSF is enhanced 2.5-fold by the addition of either stem cell factor or flk-2/flt-3 ligand, and is enhanced five-fold by a combination of these factors. Autologous serum from patients in recovery phase after high-dose chemotherapy should be used instead of fetal calf serum or human donor pooled AB serum for the reasons explained in [5].

Table 3. Inducers used to convert human myeloid cells into dendritic cells

Material source	Starting population	Cytokine or inducer	Resulting phenotype	Functional activities
Blood	MO	IL-1 and IL-6	Indeterminate MO/MOAC	Allogeneic T cell stimulation
Blood	MO	3% fetal calf serum	MOAC/LC$^-$ like cell (CD1a$^-$/CD14$^-$	Phagocytosis low, MLR$^-$
Blood	MO	IL-4 and GM-CSF	AC (CD1$_2^-$)	*Mycobacterium*-specific-T cell proliferation
Blood	MO	IL-4 and γ-IFN	DC (CD14$^-$)	MLR$^-$
Blood	MO	IL-4 and GM-CSF	DC (CD14)	MLR$^-$
Blood	MO	IL-4 and GM-CSF	MODC	MLR$^-$
Blood	MO	IL-4, GM-CSF and γ-IFN	MODC	Phagocytosis$^-$, MLR$^-$
Cord blood	CD34$^+$ cells	TNF-α and GM-CSF	DC	MLR$^-$
Cord blood	CD34$^+$ cells	TNF-α, GM-CSF, and CD40L	DC	MLR$^-$
Bone marrow (rat)	Nucleated bone marrow cells	IL-3 (low), M-CSF (low), linoleic acid, α tocopherol and cholecalciferol	DC	Allogeneic T cell stimulation, phagocytosis
Bone marrow	CD34$^+$ cells	TNF-α, GM-CSF, and IL-3	DC	ND
Bone marrow	CD34$^+$ cells	TNF-α, GM-CSF, and SCF	DC	Phagocytosis, MLR
Blood	CD33$^+$/CD14 cells	None	DC	MLR$^-$
Blood	Adherent cell (MO)	IL-4 and GM-CSF	DC	MLR$^-$
Blood	PBMC	IL-4 and GM-CSF	DC	MLR$^-$
Mobilized blood	CD34$^+$ cells	TNF-α$^-$ GM-CSF, SCF, and FL	DC	MLR^{++}, melanoma peptide presentation, xenogenic antigen presentation

AC = accessory cell; DC = dendritic cell; GM-CSF = granulocyte-macrophage colony-stimulating factor; γ-IFN = γ-interferon; IL = interleukin; LC = Langerhans cell; M-CSF = macrophage colony-stimulating factor; MLR = mixed leukocyte reaction; MO = monocyte; MOAC = monocyte-derived accessory cell; MODC = monocyte-derived dendritic cell; ND = not determined; PBMC = peripheral blood mononuclear cell; SCF = stem cell factor; TNF-α = tumor necrosis factor-α; FL = flk-2/flt3 ligand.

Summary and Future Directions

Since dendritic cells have been shown to be intimately involved in the generation of CD4$^+$ and CD8$^+$ T lymphocyte-mediated tumor-specific immunity, it is attractive to speculate that vaccination with these cells pulsed or engineered ex vivo to present tumor antigens may be effective in generating tumor immunity in vivo. Among the potential sources of dendritic cells, peripheral blood is certainly the richest and most accessible source in patients with cancer, but it remains to be confirmed if there are functional differences that will favor the use of monocyte- or CD34$^+$-derived dendritic cells. Our group has envisioned a therapeutic protocol for primary cancer treatment combining intensive chemotherapy supported by recombinant hematopoietic growth factors, generation of dendritic cells from CD34$^+$ mobilized cells, engineering of the dendritic cells to present tumor antigens, and vaccination with the engineered dendritic cells. It remains to be established if the vaccination could be enhanced by the use of adjuvant cytokines, such as rHuGM-CSF and/or flk-2/flt-3 ligand, as has been seen in model systems [31-33].

Acknowledgment

This work was partially supported by the Associazione Italiana Ricerca Cancro (AIRC).

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